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ORIGINAL ARTICLE

Dietary polyunsaturated fat intake is associated with low-density lipoprotein size, but not with susceptibility to oxidation in subjects with impaired glucose metabolism and type II diabetes: the Hoorn study

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Objective: A high monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) intake is associated with lower plasma low-density lipoprotein (LDL)-cholesterol. However, PUFA may increase the susceptibility of LDL to undergo oxidative modifications. The aim of this study was to analyze the association of habitual dietary fat intake with LDL size and oxidizability.
Design: Cross-sectional.
Setting: Cohort study.
Subjects: Seven hundred and fifty-eight subjects with normal, impaired glucose metabolism and type II diabetes.
Interventions: Mean LDL size was measured by high-performance gel-filtration chromatography. *In vitro* oxidizability of LDL was determined by measuring lag time, reflecting the resistance of LDL to copper-induced oxidation. Information about dietary fat intake was obtained by a validated food frequency questionnaire.
Results: PUFA intake (energy percent) was significantly and negatively associated with LDL size in subjects with type II diabetes (standardized beta (95% confidence interval) −0.17 (−0.28;−0.06)) and impaired glucose metabolism – although not statistically significant – (−0.09 (−0.24;0.05)), but not in subjects with normal glucose metabolism (0.01 (−0.10;0.12)) (*P*-value for interaction = 0.02). No significant associations were observed for total, saturated fat and MUFA intake with LDL size. Intake of fat was associated with lag time; however, the small magnitude of the associations suggested that the composition of dietary fat is not a major factor affecting lag time. The same association with lag time was observed in all three glucose metabolism categories.
Conclusions: In individuals with abnormal glucose metabolism, higher PUFA intake is associated with smaller LDL particle size, but does not alter the susceptibility of LDL to *in vitro* oxidation.
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Keywords: Low-density lipoprotein cholesterol; oxidation; type II diabetes; glucose tolerance

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Introduction

Small, dense low-density lipoprotein (LDL) particles are associated with a higher risk of atherosclerosis (Gardner

et al., 1996; Lamarche *et al*., 1997). Oxidation of LDL is considered to be the key event in the development of atherosclerosis (Steinberg *et al*., 1989). Small, dense LDL is more susceptible to oxidation (de Graaf *et al*., 1991; Chait *et al*., 1993; Dejager *et al*., 1993). Subjects with impaired glucose metabolism and type II diabetes have an adverse lipid profile, including predominance of small, dense LDL (Austin *et al*., 2000; Yoshino *et al*., 2002), and they have an increased risk of atherosclerosis (Kannel and McGee, 1979; de Vegt *et al*., 1999).

Previous research has demonstrated that there is a relationship between dietary fat intake and the incidence of atherosclerosis (Schaefer, 2002). Monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) intake are associated with a decrease in plasma LDL-cholesterol (Vega *et al*., 1982; Rivellese *et al*., 2003). However, PUFA may increase the susceptibility of LDL to undergo oxidative modifications (Scheffer *et al*., 2001; Kratz *et al*., 2002). It has previously been found that dietary PUFA intake reduced LDL size (Kratz *et al*., 2002). The aim of this study was to investigate the association of fat intake with LDL size and susceptibility to *in vitro* oxidation of LDL in a large population cohort. In addition, we investigated whether these possible associations were similar in subjects with normal glucose metabolism, impaired glucose metabolism and type II diabetes.

Subjects and methods

Study design and methods

The Hoorn study, a cohort study on glucose metabolism and cardiovascular risk factors among a general population in the Netherlands, started in 1989 with 2484 subjects aged 50–75 years at baseline (Mooy *et al*., 1995). In 2000–2001, a follow-up examination was conducted in selected subjects, then aged 60–87 years. We invited all surviving subjects with type II diabetes ($n=176$), and random samples of individuals with normal glucose metabolism ($n=705$) or impaired glucose metabolism ($n=193$) based on their glucose metabolism status at the previous examination in 1996–1998 (de Vegt *et al*., 2001). Of the 1074 individuals invited for the 2000–2001 follow-up examination, 648 subjects participated (60.3%). Among the reasons for not participating in the follow-up examinations were lack of interest (30%), comorbidity (23%), high age (7%), unwillingness to travel (6%), participation considered too time consuming (6%) and miscellaneous reasons (15%). Thirteen percent were complete non-responders. To increase the number of individuals with type II diabetes, we also invited 217 individuals with type II diabetes from the Hoorn Screening study aged 50–75 years, carried out in 1999, of which 182 participated (Spijkerman *et al*., 2002). The Ethical Review Committee of the VU University Medical Center approved the study. All subjects gave their written informed consent before participation.

Anthropometric data were obtained from all subjects, wearing light clothes only. Body mass index (BMI) was calculated by dividing weight by height square. On their visit to the research center, participants were asked to bring their medication. Name and dosages of medications were recorded and coded. Cardiovascular history and lifestyle habits, including smoking, were assessed by questionnaire.

HaemoglobinA1c was analyzed by high-performance liquid chromatography (reference range 4.3–6.1%). Fasting plasma glucose concentration and 2-h post-load plasma glucose concentration, total cholesterol, high-density lipoprotein (HDL)-cholesterol and triglyceride were measured by enzymatic methods (Roche, Mannheim, Germany). LDL-cholesterol was directly determined by the N-geneous assay (Genzyme, Cambridge, MA, USA). Insulin was determined using a two-site immuno-radiometric test. Paired monoclonal antibodies were used (Medgenix, Diagnostics, Fleurus, Belgium).

LDL size and oxidizability

LDL was prepared by ultracentrifugation of ethylenediaminetetraacetic acid (EDTA)-plasma densities between 1.019 and 1.063 kg/l. After isolation, LDL samples were kept under nitrogen at 4°C in the dark. The mean LDL size was measured by high-performance gel-filtration chromatography as described previously (Scheffer *et al*., 1997), with thyroglobulin and fibrinogen as calibrators of known diameter. Intra-assay and inter-assay coefficients of variation for the gel-filtration chromatography method were 0.1 and 0.2%, respectively.

LDL samples were desalted by gel-filtration to remove excess of EDTA using 5 ml HiTrap desalting columns (Amersham Bio-sciences, Uppsala, Sweden), equilibrated with 10 mmol/l phosphate-buffered saline (pH 7.4), containing 2.8 μmol/l EDTA. *In vitro* oxidizability of LDL was determined by measuring the resistance of LDL to oxidation and was expressed as lag time (Esterbauer *et al*., 1989). Experiments were performed at 30°C on a HTS7000 plate reader (Perkin-Elmer, Norwalk, CT, USA). Copper (II) ions were used as pro-oxidant at a final concentration of 18 μmol/l. The intra-assay and inter-assay coefficients of variation for lag time determination were 1.6 and 3.6%, respectively. All samples were assayed for LDL *in vitro* oxidizability within 2 days of LDL isolation. The total amount of conjugated dienes formed during *in vitro* oxidation was calculated from the difference between the maximum and initial absorbance at 232 nm using 29 500 l/mol/cm as molar extinction coefficient (Kleinveld *et al*., 1992).

Dietary intake

Information about dietary intake of the participants was obtained by a validated food frequency questionnaire (Ocke *et al*., 1997a, b), which was linked to an extended version of the computerized Dutch Food composition table 1996 (Stichting NEVO, 1996). Total fat intake was split up in

saturated fat intake, MUFA and PUFA intake. Fat intake was defined as energy percentages ((fat (kcal) /total energy (kcal)) *100) to correct for total energy intake.

Statistical analysis

Three glucose metabolism categories were defined according to the WHO99 criteria (Alberti and Zimmet, 1998). We excluded people with missing data on primary variables of interest (LDL size and lag time and conjugated diene production $n=43$, glucose metabolism $n=14$ and food questionnaire $n=16$). We also excluded one individual with extreme values of dietary intake (energy intake $>50\,000$ kJ). Thus, the study population consisted of 758 individuals, 281 subjects with normal glucose metabolism, 179 subjects with impaired glucose metabolism and 298 subjects with type II diabetes. Baseline characteristics are presented as means with standard deviations (s.d.), but for insulin and triglyceride concentrations (skewed data) the median and interquartile ranges are shown. Univariate Pearson's correlations were calculated between explored variables and outcomes. To test for linear trend in study sample characteristics over categories of glucose metabolism, a linear regression model was used with glucose metabolism category as a linear explanatory variable. We tested for possible effect modification by glucose metabolism or sex on the association of fat intake with lag time and LDL size by calculating the respective interaction terms. Linear regression models were performed stratified for glucose metabolism with lag time and LDL size as dependent variable and with independent variables of interest (amount and type of dietary fat defined as energy percentage), first adjusted for sex and age (model 1), and subsequently for other macronutrients (carbohydrates, protein) (model 2), triglycerides (model 3 LDL size), and vitamin E supplement use, LDL-cholesterol, HDL-cholesterol, and triglycerides, LDL particle size, and HbA1c (model 3 lag time). All associations of the regression analyses are reported as standardized betas with 95% confidence intervals. Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS) for Windows version 10.1.

Results

Table 1 presents the characteristics and the dietary (fat) intake of men and women stratified for glucose metabolism. LDL size was smaller in men than in women ($P<0.001$). The mean LDL size of subjects with type II diabetes was smaller than in subjects with normal and impaired glucose metabo-

Table 1 Characteristics of men and women stratified by glucose metabolism status ($n=758$)

	Men			*Women*		
	NGM	*IGM*	*Diabetes*	*NGM*	*IGM*	*Diabetes*
N	136	92	152	145	87	146
Age (years)	69 (6)	70 (7)	67 (9)	68 (6)	71 (6)	69 (8)
LDL size (nm)	21.5 (0.3)	21.4 (0.4)	21.2 (0.5)*	21.8 (0.3)	21.6 (0.4)	21.4 (0.5)*
LDL lag time (min)	72.2 (9.8)	71.1 (9.1)	71.3 (9.1)	72.5 (9.5)	70.8 (9.4)	70.3 (9.8)
Conjugated diene production (μmol/g LDL protein)	609 (69)	600 (82)	609 (81)	624 (76)	602 (72)	601 (70)*
Fasting glucose (mmol/l)	5.47 (0.37)	6.07 (0.48)	7.77 (1.83)[a]	5.39 (0.38)	6.09 (0.48)	7.59 (1.65)[a]
Post-load glucose (mmol/l)	5.45 (1.17)	7.88 (1.89)	11.37 (3.12)[a]	5.76 (1.13)	8.13 (1.51)	11.93 (2.68)[a]
HbA1c (%)	5.7 (0.4)	5.8 (0.4)	6.6 (0.9)	5.7 (0.4)	5.9 (0.3)	6.6 (0.9)
Insulin (pmol/l)[b]	47 (37–63)	62 (45–78)	83 (52–119)*	45 (35–56)	75 (55–93.75)	87 (61–113)*
Total cholesterol (mmol/l)	5.4 (1.0)	5.5 (1.0)	5.2 (1.0)	6.2 (0.9)	6.0 (1.0)	5.9 (1.0)
HDL-cholesterol (mmol/l)	1.30 (0.35)	1.27 (0.32)	1.14 (0.3)	1.70 (0.40)	1.59 (0.42)	1.36 (0.35)*
LDL-cholesterol (mmol/l)	3.5 (0.8)	3.7 (0.9)	3.4 (0.9)	3.9 (0.9)	3.8 (0.9)	3.7 (0.9)
Triglycerides (mmol/l)[b]	1.3 (0.9–1.6)	1.3 (1.0–1.7)	1.5 (1.1–2.2)*	1.1 (0.8–1.5)	1.3 (1.0–1.7)	1.6 (1.2–2.3)*
Body mass index (kg/m^2)	26.2 (3.3)	27.2 (3.2)	28.6 (3.5)*	26.0 (3.3)	28.6 (4.8)	29.5 (5.2)*
Smoking (%)	20	22	14	11	14	11
Lipid-lowering drugs (%)	15	15	23	12	17	18
Energy (kJ)	9081 (2010)	8786 (2036)	9111 (2606)*	7502 (2336)	7048 (1759)	7138 (2038)
Carbohydrate (energy percent)	45.6 (6.2)	42.9 (6.7)	43.2 (6.7)*	46.2 (6.4)	47.2 (7.5)	46.7 (6.4)
Protein (energy percent)	14.7 (2.3)	14.6 (1.7)	15.5 (2.4)	15.5 (2.7)	15.8 (2.5)	16.1 (2.6)
Total fat (energy percent)	34.5 (5.6)	36.1 (6.1)	35.2 (5.2)	34.4 (5.4)	33.0 (6.2)	35.0 (5.5)
Saturated fat (energy percent)	14.6 (2.8)	15.1 (2.9)	14.4 (2.4)	14.9 (3.0)	14.2 (2.8)	14.9 (2.9)
MUFA (energy percent)	12.9 (2.5)	14.0 (3.0)	13.5 (2.4)	13.1 (2.6)	12.4 (2.8)	13.0 (2.6)
PUFA (energy percent)	6.6 (2.5)	6.7 (2.5)	7.0 (2.0)*	6.0 (1.8)	6.2 (1.8)	6.7 (2.2)*
Vitamin E (%)	6.6	5.4	3.3	4.1	5.7	3.4
Multivitamin (%)	11.8	5.4	8.6	17.2	10.3	12.3

Abbreviations: IGM, impaired glucose metabolism; MUFA, monounsaturated fat; NGM, normal glucose metabolism; PUFA, polyunsaturated fat.
*P for trend (age-adjusted) <0.05.
[a]$P<0.05$ by definition.
[b]Baseline characteristics are presented as means with s.d.'s, but for insulin and triglyceride concentrations (skewed data) the median and interquartile ranges are shown.

lism ($P<0.001$ for both men and women). No significant differences were observed in lag time between men and women, nor between glucose metabolism categories. The mean energy intake was lower in women than in men ($P<0.001$). There was no significant difference in type of fat intake between men and women and between the three categories of glucose metabolism.

In Table 2, univariate Pearson's correlations are shown between dietary fat intake and outcome variables. Weak correlations were shown between total fat, saturated fat and MUFA with LDL size, lag time and conjugated diene production. There was a strong negative correlation between PUFA and LDL size, and a strong positive correlation between PUFA and conjugated dienes.

Table 2 Pearson's correlations of dietary fat intake with LDL size, lag time and conjugated dienes

	LDL size (nm)	*Lag time (min)*	*Conjugated dienes (μmol/g)*
Total fat (energy %)	−0.081	−0.070	0.032
Saturated fat (energy %)	0.033	−0.067	−0.092
MUFA (energy %)	−0.077	−0.074	0.000
PUFA (energy %)	−0.165	−0.001	0.210

Abbreviations: LDL, low-density lipoprotein; MUFA, monounsaturated fat; PUFA, polyunsaturated fat.

Men and women were pooled in the linear regression analyses, because the relation between dietary fat and LDL size or lag time was not modified by sex ($P>0.10$). Table 3 shows the linear regression analyses of the total population for LDL size as dependent variable, adjusted for age and sex. After stratification for glucose metabolism status, a significant negative association was observed for PUFA intake and LDL size in subjects with type II diabetes. This association was less strong in subjects with impaired and absent in subjects with normal glucose metabolism (Table 3) (P for interaction = 0.02). The association between PUFA intake and LDL size was not explained by carbohydrate and protein intake. Adjustment for triglyceride did not alter the results. There were no significant associations between total fat, saturated fat, MUFA intake and LDL size.

Intake of total fat, saturated fat and MUFA was negatively associated with lag time with standardized betas of −0.07 (−0.14; 0.0002), −0.07 (−0.14; 0.0002) and −0.07 (−0.14; −0.0027), respectively. The associations remained but lost significance after adjustment for carbohydrate and protein intake. Adjustment for vitamin E, the serum concentrations of LDL-cholesterol, HDL-cholesterol, and triglycerides, LDL particle size, and glycemic control further attenuated these associations with standardized betas of −0.02 (−0.11; 0.07) for total fat, −0.02 (−0.10; 0.06) for saturated fat and −0.03 (−0.12; 0.06) for MUFA. No significant differences between glucose metabolism categories were observed in associations

Table 3 Age- and sex-adjusted associations of dietary fat intake with LDL size stratified for glucose metabolism status

	NGM (n = 281)	*IGM (n = 179)*	*Diabetes (n = 298)*	*Overall*
	Standardized beta (95% CI)			
Total fat (energy percent)				
Model 1	−0.04 (−0.15; 0.06)	0.04; (−0.11 0.19)	−0.09 (−0.20; 0.02)	−0.06 (−0.12; 0.01)
Model 2	0.01 (−0.12; 0.15)	0.02; (−0.17 0.20)	−0.01 (−0.16; 0.13)	−0.01 (−0.10; 0.08)
Model 3	−0.04 (−0.13; 0.05)	0.02; (−0.10 0.15)	−0.05 (−0.13; 0.03)	−0.03 (−0.08; 0.02)
Saturated fat (energy percent)				
Model 1	−0.05 (−0.15; 0.06)	0.13 (−0.01; 0.28)	0.00 (−0.11; 0.11)	0.02 (−0.05; 0.09)
Model 2	0.00 (−0.13; 0.12)	0.14 (−0.02; 0.30)	0.08 (−0.04; 0.21)	0.08 (0.00; 0.15)
Model 3	−0.05 (−0.14; 0.04)	0.13 (0.01; 0.24)	0.01 (−0.07; 0.10)	0.03 (−0.03; 0.08)
MUFA (energy percent)				
Model 1	−0.05 (−0.16; 0.06)	0.01 (−0.14; 0.16)	−0.06 (−0.17; 0.05)	−0.04 (−0.11; 0.03)
Model 2	0.01 (−0.13; 0.15)	−0.02 (−0.21; 0.16)	0.05 (−0.10; 0.20)	0.02 (−0.07; 0.11)
Model 3	−0.06 (−0.14; 0.03)	0.01 (−0.12; 0.13)	−0.01 (−0.09; 0.07)	−0.01 (−0.07; 0.04)
PUFA (energy percent)				
Model 1	0.02 (−0.10; 0.14)	−0.09 (−0.24; 0.05)	−0.17 (−0.28; −0.06)	−0.13 (−0.20; −0.06)
Model 2	0.03 (−0.10; 0.15)	−0.11 (−0.26; 0.04)	−0.14 (−0.26; −0.03)	−0.12 (−0.19; −0.05)
Model 3	0.04 (−0.06; 0.14)	−0.11 (−0.23; 0.00)	−0.13 (−0.21; −0.05)	−0.09 (−0.14; −0.04)

Abbreviations: IGM, impaired glucose metabolism; MUFA, monounsaturated fat; NGM, normal glucose metabolism; PUFA, polyunsaturated fat.
Model 1 = Age and sex adjusted.
Model 2 = Model 1 + carbohydrate and protein intake.
Model 3 = Model 1 + triglyceride.
A standardized beta of –0.17 indicates that if the independent variable (PUFA intake) decreases 1 s.d., the dependent variable (LDL size) increases 0.17 s.d. This corresponds with a of beta of –0.04, indicating that if PUFA intake increases with 1 energy percent, LDL size decreases with 0.04 nm in subjects with type II diabetes. A standardized beta of –0.13 corresponds with a of beta of –0.03, indicating that if PUFA intake decreases with 1 energy percent, LDL size increases with 0.03 nm.

of fat intake with lag time (data not shown). Saturated fat was negatively, and PUFA was positively associated with the total amount of conjugated dienes formed during oxidation of LDL (−0.09 (−0.17; −0.02) and 0.22 (0.15; 0.29), respectively), and these associations were independent of carbohydrate and protein intake (data not shown).

All associations are presented as standardized betas. For example, a standardized beta of −0.12 indicates that if the independent variable (PUFA intake) increases 1 s.d., the dependent variable (LDL size) decreases 0.12 s.d.

Discussion

In this study, we observed that habitual dietary PUFA intake was significantly and negatively associated with LDL size in subjects with type II diabetes, but less strong in subjects with impaired and not at all in subjects with normal glucose metabolism, indicating that the relationship between PUFA and LDL size is modified by glucose metabolism status. There was no association of PUFA intake with lag time.

We used the automated gel-filtration chromatography method because of good reproducibility, high precision and the possibility to analyze large series of samples. We used *in vitro* measures for LDL oxidizability, because lag time has been found to be inversely related to progression of coronary atherosclerosis (Regnstrom *et al*., 1992). The suitability of *in vitro* susceptibility to oxidation may be questioned, as we recently observed that this measure does not correlate with *in vivo* LDL oxidation (Scheffer *et al*., 2003).

The reproducibility and validity of the food frequency questionnaire has been evaluated previously, and was generally good (Ocke *et al*., 1997). The energy percentages for the different types of fat were higher than recommended by the WHO dietary recommendations for the prevention of chronic disease (Shikany and White, 2000).

The food frequency questionnaire that was used has limitations in capturing energy intake (Schatzkin *et al*., 2003). The Observing Protein and Energy Nutrition data indicate that a true relative risk between absolute intakes of dietary factors with disease would be attenuated. This attenuation is lessened in analyses of energy-adjusted factors, which were reported in our study. Nevertheless, if this limitation is applicable to beta's also, it could be that the associations between habitual dietary fat intake and LDL size and oxidizability were underestimated. So the interpretation of these findings should be with caution.

To our knowledge, this is the first population cohort study to assess the association between habitual dietary fat intake and lag time and LDL size in subjects with normal and impaired glucose metabolism, and type II diabetes. The design was such that we over sampled subjects with impaired glucose metabolism and type II diabetes, to enhance the power of the study. The associations we found were weaker than anticipated. This may be explained by several factors. Firstly, the people who know that they have type II diabetes may have changed to a healthier diet. However, there was no difference in dietary fat intake between the three glucose metabolism categories. Importantly, the majority of the diabetic subjects was newly detected, and they were unaware of their diabetic state when they filled in the food frequency questionnaire. Secondly, subjects with a high BMI usually underestimate their total energy intake compared to subjects with low BMI (Braam *et al*., 1998). As subjects with type II diabetes more frequently have a high BMI than subjects with normal glucose metabolism, we adjusted for this difference and we used energy percentages of the different types of dietary fat. When we analyzed the absolute dietary fat intake (g/day), this did not show associations with lag time and LDL size (data not shown).

The associations between dietary fat and LDL size have previously been investigated in different populations. Lower total fat intake was associated with smaller LDL size in healthy non-smoking men (Dreon *et al*., 1998). Kratz *et al*.(2002) concluded that dietary MUFA and PUFA had a similar effect on LDL size in 56 healthy students (men and women). They demonstrated that an LDL size reduction occurred when replacing saturated fat by unsaturated fat, which was in line with our findings. Several studies reported that a high saturated fat intake was associated with a higher proportion of larger and less atherogenic LDL particles and with LDL size, but with a concomitant increase of the LDL-cholesterol concentration (Campos *et al*., 1995; Dreon *et al*., 1998). Rivellese *et al*. (2003) observed no effect of dietary saturated fat, MUFA and n-3 fatty acids on LDL size in 162 healthy subjects.

It has been suggested that the possible association between LDL size and dietary fat may be explained by plasma triglyceride, the strongest determinant of LDL size (Dreon *et al*., 1999). Insulin-resistant subjects, such as people with impaired glucose metabolism and type II diabetes are exposed to a longer post-prandial triglyceride excursion (Heine and Dekker, 2002). This might explain why LDL size could be more influenced by dietary fat in subjects with type II diabetes and impaired glucose metabolism than in subjects with normal glucose metabolism.

In our study, we observed a positive association between habitual intake of carbohydrate and LDL size. This is not in line with Siri and Krauss, 2005, who recently showed that increased carbohydrates, through increasing plasma triglyceride, are associated with a decline in LDL diameter. However, it must be emphasized that these results are based on a cross-sectional study design, rather than a dietary fatty acid intervention. It could be that these relationships are not strong enough to detect in a population study with normal ranges of habitual fat and carbohydrate intake.

Fatty acids have been demonstrated before to be the main determinants of LDL oxidation in a diabetic population (Scheffer *et al*., 2001). Several studies have reported the relation between dietary monounsaturated fat intake and resistance of LDL to oxidation (Reaven *et al*., 1991; Abbey

et al., 1993). The main picture that emerges from these studies is that diets rich in PUFA result in LDL that are more readily oxidized than LDL isolated form subjects who consume diets rich in MUFA. Dietary saturated fat adversely affect plasma lipids as well as susceptibility of LDL to oxidation. However, this was not observed in our study and a recent study reported that replacing saturated fat by MUFA and PUFA in low fat diets did not affect LDL oxidative susceptibility as measured by lag time (Yu-Poth *et al.*, 2000). Thus, the interaction of dietary fat with LDL composition and oxidizability is still poorly understood (Kratz *et al.*, 2002). The lack of effect of dietary MUFA on lag time might be put into the right context considering the major food sources of fatty acids in this cohort. Milk/dairy products, meat and meat products, grain and grain products and sauces contribute to fat intake in the Netherlands (Hulshof *et al.*, 2004). In such populations that consume little amounts of MUFA-rich oils or fats, intakes of MUFA are highly correlated with intakes of saturated fat because meat and meat products are relevant sources of dietary MUFA.

In the present study, we could not confirm beneficial effects of habitual MUFA intake. We observed that higher PUFA intake was associated with small LDL size and higher conjugated diene production. In the study by Scheffer *et al.* (2001), linoleic acid and oleic acid were major determinants of the amount of conjugated dienes during LDL oxidation, whereas fatty acids with three or more double bonds were negatively associated with LDL lag time. Further studies may investigate the association of the intake of individual PUFA with LDL size and oxidizability.

In conclusion, in this cross-sectional study in 758 elderly subjects, in subjects with abnormal glucose metabolism, a higher habitual PUFA intake is associated with smaller LDL size but not with lag time. Considering the major differences in metabolic effects, the type of PUFA, and n-3 versus n-6, need to be taken into account in future studies.

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